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Published in:
Oecologia

DOI:
10.1007/BF00329767

Oecologia (1990) 84:404–410

Oecologia

Within-population variability in morphology and life history of *Plantago major* L. ssp. *pleiosperma* Pilger in relation to environmental heterogeneity

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Received February 26, 1990 / Accepted in revised form May 18, 1990

Summary. An attempt was made to relate variation in life-history characteristics within a population of *Plantago major* ssp. *pleiosperma* to small-scale environmental variability. At a beach plain, embanked in 1966, a mosaic environment was distinguished with spatial variability in vegetation structure as well as in nutrient availability and water content of the soil. Differences between three subsites in contemporary selection were demonstrated, e.g. in shoot morphology and allocation to reproductive tissue. The effects of nutrient supply and waterlogging on morphology and life history were studied on lines from the three subsites in a greenhouse. For most of the traits high levels of phenotypic plasticity were observed, covering almost entirely the observed phenotypic variability at the beach plain. In all treatments lines from the shrubs had, however, a higher leaf-area ratio as well as delayed flowering when compared to lines from more open subsites. In addition, in a reciprocal transplant experiment it was demonstrated that lines from the shrubs had larger shoots with e.g. broader leaves in the shady environment of the shrubs than other lines.

From the experiments no indications were obtained that lines from any subsite were especially adapted to specific levels of nutrient supply or water content of the soil. With respect to these environmental factors *P. major* ssp. *pleisoperma* might occur and reproduce at all subsites by means of phenotypic plasticity, e.g. in plant form. However, it is suggested that spatial variability in vegetation structure caused a population subdivision in allocation patterns, leaf form and life history at the beach plain, over distances of about 15–25 m. This differentation occurred during primary succession over a period of twenty years.

Key words: Selection – Morphology – Life-history characteristics – Phenotypic plasticity – Genetic differentiation

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This study is part of a research project on variability in life-history traits within a population of *Plantago major* L. ssp. *pleiosperma* Pilger on a former beach plain. At this site a mosaic environment was distinguished with spatial variability in vegetation structure, as well as in nutrient availability and water content of the soil (Lotz and Olff 1988; Lotz and Spoormakers 1988; Troelstra et al. in press). *P. major* ssp. *pleiospermia* plants demonstrate a pronounced phenotypic variaton in plant biomass and reproductive characteristics (Lotz 1989). The main objective of the present paper is to indicate whether within-population differences in life-history characteristics and in plasticity in these properties could have evolved as an adaptation to small-scale environmental variability. The following questions will be addressed:

1. Do three subsites within this beach plain differ in contemporary selection acting on morphology and life-history of *P. major* ssp. *pleiosperma*?

2. Can genetic variation in these characteristics between plants from different subsites be demonstrated?

3. Can this genetic variation, if present, be explained by spatial heterogeneity in abiotic rather than in biotic factors?

4. At what spatial scale does genetic differentiation in morphological and life-history characteristics occur?

To answer these questions vegetative and reproductive characteristics of lines of *P. major* ssp. *pleiosperma* from the three subsites were studied in a reciprocal transplant experiment and in a greenhouse experiment with nutrient supply and water content of the substrate as experimental factors.

Materials and methods

Study sites and derivation of lines

The investigated population of *P. major* L. ssp. *pleiosperma* Pilger is located at a former beach plain on the south-west coast of the Netherlands. This beach plain was embanked in 1966. The study site is an area of about 2000 m^2. Due to differences in micro-relief, nutrient availability (Lotz and Spoormakers 1988, Troelstra et al.

in press) and vegetation structure (Lotz and Olff 1988) a mosaic environment with three subsites can be distinguished. Subsite 1 is a low-lying area; the soil is low in nutrients and frequently water saturated. The nitrogen mineralization potential (NMP), determined in the laboratory on intact soil cores after nine weeks of incubation, was relatively low (ca. 35 mg $N.L^{-1}$ of soil). This area is flooded after heavy rain and during winter; the vegetation is low (<0.05 m) and very open. Subsite 2 consists of small elevations (0.5–1.5 m in diameter) spread over subsite 1; these patches are covered with a short, dense, grassy vegetation; the soil is relatively high in nutrients. The nitrogen mineralization potential was also relatively high (NMP = 130 mg $N.L^{-1}$ of soil). Subsite 3 consists of relatively large patches (20–40 m in diameter), covered by shrubs of *Hippophae rhamnoides* L. At this subsite nutrient availability is also higher (NMP = 95 mg $N.L^{-1}$ of soil) and water content is lower than in the soil of subsite 1; plants of *P. major* ssp. *pleiosperma* are shaded by the shrubs.

In autumn 1984, 10 reproductive plants were randomly sampled from each subsite, defoliated and transplanted into pots with potting compost. The plants, which were watered daily, were grown until reproduction in a greenhouse (20° C, light-dark period: 16–8 h). Cross-pollination was prevented, while the plants flowered, by paper bags. Lines were obtained by collecting mature seeds from each plant separately.

Reciprocal transplant experiment

On 25 April 1985, seeds of 10 lines from each subsite were sown in moist dune sand in a greenhouse (20° C). At least 95% of the seeds of each line germinated within three days. On day 20 after sowing differences in dry weight of the shoots of seedlings of lines from different origins (subsites) were not significant (nested analysis of variance, $F=1.7$, $df=2, 17$, $P=0.21$). On this day half of the seedlings from each line were exposed for three days to outdoor conditions. Next, outdoor seedlings from each line were randomly selected and transplanted – roots nearly free of soil – into a randomized complete block design; ten square plots (blocks) were spread over each subsite with a single replicate of each line per plot (total number of plants: $3 \times 3 \times 10 \times 10 = 900$). Within each plot plants were positioned in a 0.10×0.10 m^2 grid. The resulting plant density is within the ranges of naturally occurring adult plants observed at each subsite. One week after transplanting mortality of the transplants was very low (<1%). On this day dead seedlings were replaced. Survivorship was recorded in 1985 on 5 and 29 June, 15 August, 1 October, in 1986 on 21 May, 12 August, 9 September, and 10 October, and in 1987 on 20 May, 10 September, and 12 October. *P. major* is a species with a rosette growth form. On 1 October 1985 the following characteristics of the surviving plants were determined: the number of leaves (N), the length (L) and the width (W) of the largest leaf (further referred to as leaf length and leaf width, respectively). The estimated leaf area (the product N*L*W) is highly correlated with the dry weight of the leaves (Lotz and Blom 1986). In October 1985 and in September and October 1986 (and 1987) mature spikes were harvested and counted. Dry weights of these spikes (including scapes) and number of seeds per plant were determined.

Greenhouse experiment

On day 20 after sowing seedlings were randomly selected from the remaining stocks (i.e. plants that were not exposed to outside conditions) of six separate lines from each subsite and planted into pots with quartz sand (height of pot 9 cm, contents 0.42 l, one plant per pot). The seedlings were sprayed regularly with water for three days after the planting, to minimize transplant effects. Three nutrient levels and two water levels were applied, together forming a full factorial treatment combination. Nutrient levels were created by adding different quantities of a solution twice the strength of the solution described by Steiner (1968): plants in the Low treatment received 2.6 ml solution per pot, the Intermediate treatment 38 ml, and the High treatment 75 ml. Previous experiments (Lotz and Blom 1986) generated the expectation that the biomass accumulation at these nutrient levels would be within the same range as that found at the study site. Micro-nutrients were supplied to each plant by adding 75 ml solution containing micro-nutrients with twice the concentration described by Smakman and Hofstra (1982). The high water content (treatment "Waterlogging") was created by placing the pots in flat containers with water (the water level was kept permanently at 4 cm from the top of the pots). The sand substrate was saturated with water that entered the pots through holes in the bottom. In the pots with low water content (treatment "Draining") water was added as required to maintain soil moisture at ca. 20% by weight. All pots were put in a greenhouse (20° C).

On day 36 after sowing the number of flowering plants was determined for each treatment combination. On day 46 after sowing one randomly selected plant was harvested from each line for each treatment combination. The following characteristics were measured for these harvested plants: fresh and dry weights of leaves, spikes and roots, and the leaf area. Leaf-weight ratio (LWR) was computed as the dry weight of leaves per total plant dry weight, the specific leaf area (SLA) as leaf area per dry weight leaves, and leaf-area ratio (LAR) as leaf area per total plant dry weight.

Data analysis

Survivorship curves in the field experiment were compared between subsites or origins using the Lee-Desu statistic (SPSS-subprogramme SURVIVAL, Nie and Hull 1981). When plants were alive at the last census, the interval between the time of entry into the study and the time the study ended was considered as the survival time (cf. Pyke and Thompson 1986). Differences between plants of different origins in flowering were tested by fitting a hierarchical loglinear model (Norusis 1986). Data of growth, morphology and reproductive output (non-flowering plants excluded for reproductive traits) were analysed with analysis of variance (SPSS-subprogramme MANOVA, Norusis 1986). Independent variables were for the reciprocal transplant experiment: subsite, origin and line nested within origin; for the greenhouse experiment: supply of nutrients, level of water content, origin. Non-significant mean squares were pooled with the within-cells mean squares when appropriate. Comparisons of means between origins were made by *a priori* simple contrasts. Comparisons of means over subsites or treatments were made by performing Tukey's honestly significant difference method. Because of non-normality of the total seed production per plant (non-flowering plants included), differences in this fitness component between origins were tested by Kruskal-Wallis one-way analysis of variance.

To analyse natural selection on shoot morphology and reproductive biomass at first flowering two components of fitness of transplanted seedlings were distinguished: 1. the total number of seeds per plant (non-flowering plants included) and 2. survival from the first until the second flowering. Simple regressions were performed, using both fitness components as dependent variables, and selection differentials (the covariances between relative fitness and plant characteristics) were computed. Selection differentials measure the change in the mean value of the characteristic during the selection episode within one generation (Falconer 1981). For a further discussion of techniques for quantifying selection in purely phenotypic terms without recourse to the inheritance of characteristics, see Lande and Arnold (1983), Arnold and Wade (1984).

Results

Reciprocal transplant experiment

a. Selection regimes. The *P. major* ssp. *pleiosperma* plants were on average considerably smaller at the low-lying

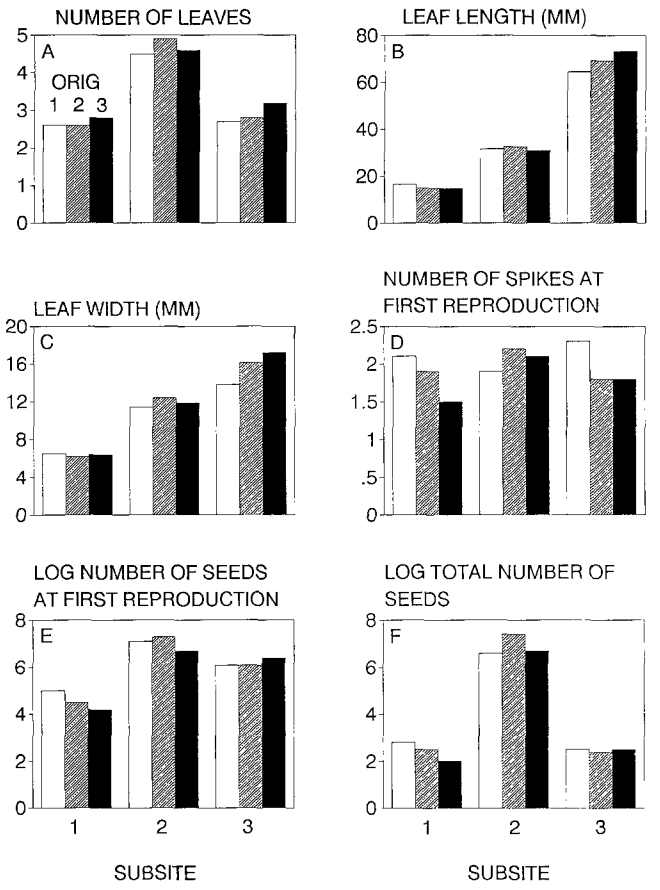

Fig. 1. Vegetative and generative characteristics of *P. major* ssp. *pleiosperma* in a reciprocal transplant experiment at a beach plain. Plants originated from three subsites: subsite 1, low-lying area with open grassland; subsite 2, patches with a dense cover of grasses; subsite 3, *Hippophae* shrub. Means were computed over ten lines: for leaf characteristics only replicates that were alive in autumn 1985 were included, and for numbers of spikes and seeds at first reproduction only replicates that flowered; the mean total number of seeds per plant was computed over the number of replicates per line at the start of the expeirment ($n=10$)

subsite 1 than at the other subsites, on day 140 after transplanting (Fig. 1 A, B and C, Table 1). These differences in plant size were associated with marked differences in selection regimes between subsites (Table 2). At subsite 1 plants were only selected for having relatively broad leaves. At this site with a low standing crop the selection differential was 0.6 mm, i.e. the mean leaf length, weighted by the relative seed yield per plant, was 0.6 mm higher than the unweighted mean (Table 2A). At subsite 3 plants were selected for having a relatively high number of broad leaves and at subsite 2 having a relatively high number of leaves that are both long and broad. At subsite 1 plants that survived the life-cycle stage between the first and second flowering had on average 0.6 mg lower dry weights of spikes at first flowering than the total group of plants that flowered at least once (Table 2B). Thus, at subsite 1, in contrast to the other subsites, plants that produced a higher biomass of spikes at the first reproduction had a lower probability of reproducing a second time.

b. Phenotypic plasticity and genetic variation. The median survival time of the transplanted seedlings was: 17.3 months at subsite 1 and 5.4 months at subsite 3. At subsite 2, from each origin more than 50% of the plants were still alive at the end of the experiment. No significant differences between origins in survival curves could be detected. Differences between origins in survival rates of transplanted seedlings until the first and the second flowering were also non-significant.

Variation in both vegetative and generative characteristics of transplanted plants could mainly be explained by subsite effects (Table 1, Fig. 1). At subsite 1 and 3 plants from all origins had fewer leaves than at subsite 2 (Fig. 1A, each contrast $P<0.01$), whereas the numbers of leaves at subsite 1 and 3 were not significantly different. Plants at subsite 1 had significantly shorter leaves than plants at subsite 2 and 3 (Fig. 1B, $P<0.05$ and $P<0.001$, respectively), and these leaves were also narrower (Fig. 1C, $P<0.01$ and $P<0.001$, respectively). In addition, plants at subsite 2 had shorter and narrower leaves than plants at subsite 3 ($P<0.01$ and $P<0.05$, respectively). The total number of seeds produced per plant was considerably higher in subsite 2 than in subsites 1 and 3 (Fig. 1F).

Analysis of the simple effects of origin within each subsite revealed that in the shade of the shrubs (subsite 3) native plants had broader leaves than plants from origin 1 (Fig. 1C, $P<0.001$). The same tendency was found for the estimated leaf area per plant ($F=2.8$; $df=4,715$; $P<0.05$). At subsite 1, plants from origin 3 had less spikes than plants from origin 2 and from origin 1 (Fig. 1D, $P<0.05$ and $P<0.001$, respectively). There seems to be a slight tendency that native plants had a higher total seed production (taken over three years, non-reproducing plants included) at subsite 1 and 2 (Fig. 1F; $P=0.14$ and $P=0.24$, respectively).

Effects of the line and subsite × line represent genetic variation not related to subsites. These effects were present for length and width of leaves and the number of the seeds at first reproduction.

Greenhouse experiment

Nutrient supply influenced all plant characteristics determined on day 46 after sowing (Table 3, Fig. 2). At the low nutrient treatment, plants had lower dry weights than plants at the intermediate and high nutrient treatments ($P<0.001$). The lower plant dry weight at the low nutrient treatment was coupled with a lower LWR ($P<0.01$) and a lower LAR ($P<0.01$). On day 36 after sowing the number of flowering plants was highest in the higher nutrient treatments (Fig. 2, $P<0.001$).

Waterlogged plants had, on average, slightly higher dry weights than plants in the drained treatment, but had a lower LWR (Table 3, Fig. 2). This lower biomass allocation to leaves of the waterlogged plants was coupled with both a lower SLA and LAR. At the treatment waterlogging differences in LWR between high nutrient plants and low nutrient plants were not significant, while at the treatment draining the LWR increased with the

Table 1. Results of nested ANOVA on characteristics of lines of *Plantago major* ssp. *pleiosperma* at October 1985 (**A**) and at first reproduction (**B**) in a reciprocal transplant experiment. Lines originated from three subsites. F-values are given together with degrees of freedom (in parentheses). Levels of significance: ns not significant, $*P<0.05$, $**P<0.01$, $***P<0.001$

A.

Source of variation	Dependent variables		
	Number of leaves	Leaf length	Leaf width
Subsite	139.8 (2,715)***	246.0 (2,54)***	293.3 (2,804)***
Origin	1.4 (2,688) ns	0.8 (2,27) ns	3.2 (2,27) ns
Line	1.0 (27,661) ns	2.4 (27,661)***	1.8 (27,661)**
Subsite × Origin	2.3 (4,715) ns	1.6 (4,54) ns	4.7 (4,804)***
Subsite × Line	1.1 (54,661) ns	2.0 (54,661)***	1.1 (54,661) ns

B.

Source of variation	Dependent variables		
	Number of spikes[1]	Dry weight of spikes[1]	Number of seeds
Subsite	1.5 (2,402) ns	147.8 (2,453)***	185.7 (2,380)***
Origin	2.3 (2,375) ns	0.5 (2,426) ns	0.5 (2,27) ns
Line	0.5 (27,348) ns	0.7 (27,399) ns	1.6 (27,328)*
Subsite × Origin	2.4 (4,402)*	1.5 (4,453) ns	1.8 (4,380) ns
Subsite × Line	0.7 (54,348) ns	1.0 (54,399) ns	1.1 (52,328) ns

[1] Analysis of variance after log-transformation

Table 2. Analysis of selection on shoot morphology and reproductive biomass at first flowering of *Plantago major* ssp. *pleiosperma* at a former beach plain. Selection differentials with respect to total seed production (**A**) and to the probability of flowering a second time (**B**) are provided. Levels of significance of the correlation coefficient between the plant characteristics at first flowering and the component of relative fitness: ns not significant, $*P<0.05$, $**P<0.01$, $***P<0.001$

	Subsite		
	1	2	3
A			
Number of leaves	0.2 ns	0.6***	1.4***
Leaf length (mm)	1.5 ns	4.5***	−6.2 ns
Leaf width (mm)	0.6*	1.3***	4.5*
B			
Biomass of the spikes at first reproduction (mg)	−0.6*	−0.1 ns	0.0 ns

nutrient supply ($P<0.05$). In the intermediate and the high nutrient treatments drained plants flowered less frequently than waterlogged plants (Fig. 2, $P<0.001$). Compared to the drained treatment, waterlogging also caused higher dry weights of spikes (Table 3, Fig. 2).

Lines from the low area (subsite 1) had over all treatments a higher dry weight than lines from the shrubs (subsite 3) (Fig. 2, Table 4). The lower plant dry weight of lines from subsite 3 was coupled with a higher SLA and, only when compared to lines from subsite 1, with a higher LAR (Fig. 2, Table 4). Differences in percentage dry matter were not significant between plants from different origins (data not presented). Both on day 36 and 46 after sowing, less plants from subsite 3 were flowering compared to plants from subsites 1 and 2 (in each case $P<0.001$). On day 46 after sowing, plants from subsite 3 had lower spike dry weights than plants from subsite 1 and 2 (Table 4).

Discussion

Analysis of selection

The present study demonstrates differences in selection regimes between specific subsites at the beach plain. With respect to shoot characteristics of *P. major* ssp. *pleiosperma* solely selection for broader leaves could be demonstrated at the low-lying area (subsite 1). The resulting relatively decumbent growth form may be adaptive in the low, very open vegetation, because either the microclimate will be more favourable at near ground level or plants with such a growth form will be less susceptible to grazing (e.g. by rabbits). On the small elevations (subsite 2) plants with many, long and broad, leaves produced more seeds. Through overtopping by means of these longer leaves *P. major* ssp. *pleiosperma* probably competes better against grasses, that grow on these patches in a dense sward. In the shrubs (subsite 3) plants were selected that had relatively more leaves, which were relatively short but broad. In contrast to subsite 2, in subsite 3 plants, irrespective of their leaf length, are always affected by shade. In this environment broad short leaves with relatively little need of support tissue may have higher photosynthetic capacity than

Table 3. Results of ANOVA for effects of nutrient supply and water content of the substrate on vegetative characteristics and reproduction of *Plantago major* ssp. *pleiosperma*. Lines originated from three origins (subsites) within a beach plain. *F*-values are given, together with degrees of freedom (in parentheses). In the analysis of spikes dry weight data from the low nutrient supply were excluded because of empty cells. Abbreviations: nut, nutrient supply; Wat, water content; Orig, origin. Levels of significance: ns not significant, $*P<0.05$, $**P<0.01$, $***P<0.001$

Source of variation	Dependent variables		
	Plant dry weight[1]	Leaf-weight ratio	Specific leaf area
Nut	170.2 (2,89)***	42.7 (2,89)***	6.2 (2,88)**
Wat	15.0 (1,89)***	8.9 (1,89)**	37.7 (1,88)***
Nut × Wat	0.7 (2,89) ns	6.4 (2,89)**	13.6 (2,88)***
Orig	5.2 (2,89)**	1.6 (2,89) ns	10.4 (2,88)***
Nut × Orig	0.4 (4,89) ns	1.1 (4,89) ns	0.4 (4,88) ns
Wat × Orig	0.1 (2,89) ns	0.6 (2,89) ns	1.6 (2,88) ns
Nut × Wat × Orig	0.4 (4,89) ns	1.1 (4,89) ns	0.6 (4,88) ns

	Leaf-area ratio	Spikes dry weight[1]
Nut	17.0 (2,88)***	14.6 (1,59)***
Wat	26.7 (1,88)***	11.7 (1.59)***
Nut × Wat	5.8 (2,88)**	0.1 (1,59) ns
Orig	5.0 (2,88)**	5.6 (2,59)**
Nut × Orig	1.0 (4,88) ns	1.3 (2,59) ns
Wat × Orig	0.5 (2,88) ns	0.1 (2,59) ns
Nut × Wat × Orig	0.7 (4,88) ns	1.4 (2,59) ns

[1] Analysis of variance was performed after log-transformation

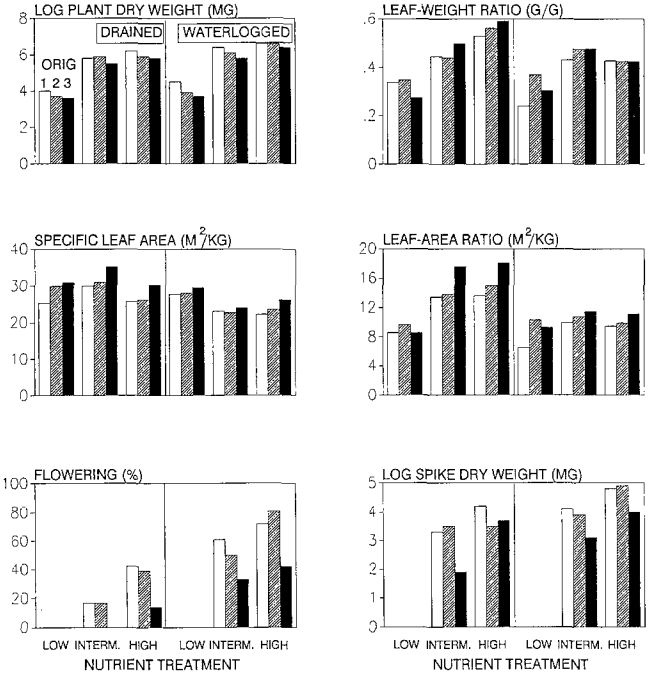

Fig. 2. The effect of nutrient supply and water saturation of the substrate on growth, morphology and flowering of *Plantago major* ssp. *pleiosperma*. Plants originated from a low-lying area (orig 1), patches with a dense cover of grasses (orig 2) and *Hippophae* shrubs (orig 3). Means were computed over six lines. For dry weight of spikes only flowering plants were included

Table 4. *A priori* contrasts between plants from three origins. Plant characteristics were selected for which effects due to origin were significant in the analysis of variance (Table 3). Means with the same letter are not significantly different ($P>0.05$)

Dependent variable	Origin means		
	1	2	3
Plant dry weight (mg)[1]	5.6^{a}	5.4^{ab}	5.1^{b}
Specific leaf area (m^2/kg)	25.6^{a}	26.8^{a}	29.3^{b}
Leaf-area ratio (m^2/kg)	10.3^{a}	11.7^{ab}	12.8^{b}
Spike dry weight (mg)[1]	3.4^{a}	3.5^{ab}	3.2^{b}

[1] Analysis of variance was performed after log-transformation

long narrow leaves (Givnish 1987). Plants with these broad leaves may, therefore, have higher reproductive outputs.

At subsite 1, in contrast to the other sites, a relatively high investment in reproductive tissue at first reproduction was correlated with a lower probability of survival to the next reproductive period. Therefore, these results show evidence of a cost of reproduction of *P. major* ssp. *pleiosperma* which is apparently only expressed in an environment in which plant growth is considerably restricted (e.g. in this specific case by low fertility and water content of the soil). Reznick (1985) also summa-

rized studies which demonstrated that costs of reproduction were only evident in environments where food availability was restricted or adults were subjected to extra stress.

Plasticity and genetic variation in relation to different selection regimes

In the reciprocal transplant experiment, variation in characteristics of morphology and life history of *P. major* ssp. *pleiosperma* was mainly due to phenotypic plasticity. Such relatively large environment components of phenotypic variation in life-history characteristics were earlier demonstrated between populations of *P. lanceolata* (Antonovics and Primack 1982) and between populations of *P. major* (Lotz and Blom 1986; Lotz in press).

At the beach plain the range of phenotypic variation in shoot dimensions and flowering was similar to that in the greenhouse, in reaction to variation in nutrient supply. It seems likely that the lower biomass of shoots at subsite 1, as compared to the other subsites, is not due to negative effects of a high water content of the substrate. In the waterlogged treatment plants had even higher dry weights than in the drained treatment. This was also found by Schat (1984) for *Plantago coronopus* and *Samolus valerandi*, species that are also abundantly present at subsite 1. This author demonstrated that both species could maintain a relatively high photosynthetic activity under waterlogged conditions, and showed a high degree of root porosity. Moreover, nutrient availability might be higher in a substrate with a high water content because of a higher transport, e.g. of P (Nye and Tinker 1977; Wild 1981), which may be relevant to plants whose growth is nutrient-limited. However, in the field soils with high water contents generally have lower soil temperatures, which fact may decrease both root activity and mineralization rates, and thus plant growth.

No indications were obtained that lines from any subsite were especially adapted to specific combinations of nutrient supply and water content of the substrate. Therefore, it is suggested that plants from the mosaic environment of the beach plain cope with variation in these abiotic factors by performing phenotypic plasticity in various morphological characteristics and reproduction (Lambers et al. 1985; Kuiper and Kuiper 1988).

However, genetic variation was present for several plant characteristics. Though the relative importance of random genetic drift can not be quantified at the present beach plain, we suggest that this specific pattern of population structure was caused by differences in selection regimes. The lower biomass of lines from subsite 3, as compared to the lines from subsite 1, was associated with a higher LAR, whereas differences in percentage dry matter between origins were not significant. So, in the greenhouse the relatively smaller plants from the subsite with the shrubs had a higher investment in photosynthetical activity area than plants from the unshaded habitat of subsite 1. This higher LAR was explained by a higher SLA of plants from subsite 3. Species (Björkman 1981; Givnish 1987) or ecotypes (Björkman and Holmgren 1963) from shady environments demonstrated higher SLA than those of exposed environments. Provided that other resources are not growth limiting, plants with relatively higher SLA should also have higher RGR, and thus a higher competitive ability, in environments with low light intensities (Björkman 1981). Indeed, in the reciprocal transplant experiment lines from origin 3 had a higher biomass of leaves, and broader leaves than lines from origin 1 and 2 in the shady environment of the shrubs. This difference in leaf form, too, may be the result of differences in selection regimes due to variation in levels of shading (see above).

In the greenhouse lines from subsite 1 and 2 flowered earlier than lines from origin 3. In the reciprocal transplant experiment plants from origin 1 and 2 did not flower in an earlier year than plants from origin 3. However, differences in time of onset of flowering within the growing season have not been studied in the field.

Small-scale differentiation within twenty years

Spatial variability in vegetation structure (i.e. light intensity at the soil surface) likely caused a population subdivision in allocation pattern, leaf form and flowering phenology of *P. major* ssp. *pleiosperma*, over distances of about 15–25 m. Of course, the development of the specific pattern of vegetation structure might, in turn, depend strongly on the spatial variation in abiotic factors. Several authors reported small-scale genetic differentiation in various plant characteristics within populations (Linhart and Baker 1973; Snaydon 1970; Snaydon and Davies 1972; Turkington and Aarssen 1984, and references cited there). Since the beach plain was embanked in 1966, the genetic structure within the present population should have become established during primary succession over a period of twenty years (probably corresponding with 5–12 generations). This period of time has the same order of magnitude as was found in the Park Grass experiment at Rothamsted, which provided evidence that microdifferentiation within the perennial grass *Anthoxantum odoratum* to soil factors evolved within 40–60 years (Snaydon 1970; Snaydon and Davies 1972).

P. major is a self-compatible, wind pollinated species with a high self-fertilization rate (Van Dijk and Van Delden 1981). Van Dijk (1985) estimated the mean gene transport per generation within a population to be only 0.11–0.35 m. Such a low level of gene flow might enhance population differentiation. Although the results of the reciprocal transplant experiment showed the slight tendency that at subsite 1 and 2 native plants had a higher total seed production, differences between origins in none of the fitness components were significant. At the beach plain, Lotz (1989) observed high mortality rates of naturally occurring seedlings soon after emergence (e.g. at subsite 3 median survival times less than one month). Selection during that early life stage, which was not quantified in the present study, may have contributed to the observed pattern in population structure.

Without human interference shrubs will expand and even trees might establish at the present study site. It is a fascinating question whether also in future local adaptation of the grassland species *P. major* will keep up with this vegetation succession.

Acknowledgements. The authors gratefully acknowledge F. Berendse, C.W.P.M. Blom, J.M.M. van Damme, S.R. Troelstra and J.W. Woldendorp for comments on earlier drafts of this manuscript. These investigations were supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organisation for Scientific Research (NWO). Grassland Species Research Group Publ. No. 139.

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